

can be eliminated by shaking the suspension vigorously for five minutes before placing it in the electrophoretic cell.

Observations made in this way, and with electrometric determination of pH,<sup>4</sup> show that there is a very definite alkaline isopotential point close to pH 13.5,—that for *B. cereus* lying at about 13.3 to 13.4 and that for *Bact. coli* at 13.6 to 13.8. Above this isopotential point the cells acquire a positive charge which increases rapidly to very high values with further increase in alkalinity.

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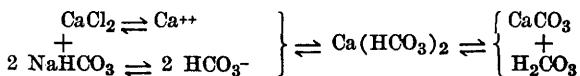
### The buffer mechanism for the calcion concentration and the determination of calcion buffer values.

By I. NEWTON KUGELMASS.

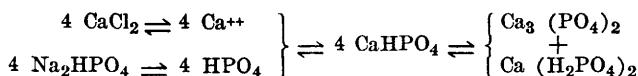
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The calcion concentration of aqueous systems is regulated by calcion buffers. They are electrolytes which resist the change in calcion concentration upon addition or removal of calcium salts. The calcion concentration is stabilized in the presence of mixtures of weak acids and their salts which react to form insoluble normal calcium salts and soluble intermediate calcium salts.

The carbonates as calcium buffers may be expressed by the buffering reactions:



The phosphates as calcium buffers may be similarly expressed:



<sup>3</sup> Winslow, C.-E. A., and Falk, I. S., *J. Bact.*, 1923, viii, 215.

<sup>4</sup> Falk, I. S., and Shaughnessy, H. J., *PROC. SOC. EXP. BIOL. AND MED.*, 1923, xx, 426.

The calcion concentration may be expressed in terms of calcion buffers by applying the law of mass action to the general form of the above buffering equations.

$$\text{Ca}^{++} = K \cdot \frac{\gamma_1 [\text{HA}]^n}{\gamma_2 [\text{BA}]^{2n}} = K \cdot \frac{[\text{HA}]^n}{[\text{BA}]^{2n}}$$

Where  $[\text{HA}]$  and  $[\text{BA}]$  are the concentrations of the calcion buffering acid and salt respectively;  $\gamma_1$  and  $\gamma_2$  their activity coefficients at the calculated ionic strength;  $n$  is the ratio of the valence of calcium to that of the acid and  $K$  is an equilibrium constant. Expressed in logarithmic units,

$$\log \frac{1}{[\text{Ca}^{++}]} = \text{pCa}$$

$$\text{and } \text{pCa} = \text{pK} + n \log \frac{[\text{BA}]^2}{[\text{HA}]}$$

This expression enables the derivation of the calcion buffer value,  $\varphi$ , the unit of which is the number of grain equivalents of calcium salt or acid necessary to change the calcion concentration one unit of  $\text{pCa}$  and is given by,

$$\varphi = \frac{d \frac{[\text{BA}]}{[\text{Ca}^{++}]}}{d \text{pCa}} = \frac{2.3}{n} \frac{K^a [\text{H}^+] [\text{O}]}{(K^a + [\text{H}^+])(K^a + 2[\text{H}^+])}$$

where  $n$  is unity for the carbonates and  $2/3$  for the phosphates.

The calcion buffer value expressed by equivalent equations shows that at any given hydrion concentration the calcion buffer value is directly proportional to the total concentration of buffer acid or salt. The calcion buffer value is independent of the nature of the weak acid provided it forms an insoluble normal calcium salt.

The calcion buffer value of the carbonates of normal blood is sum of the separate calcion buffer values.

The maximum calcion buffer value is attained when there are 0.586 parts of buffer salt and 0.414 parts of free buffer acid.

The molal calcion value at the maximum is given by the ratio  $0.395/n$  where  $n$  is unity for the carbonates and  $2/3$  for the phosphates.

The pH at which the calcion buffer value is a maximum is given by

$$\text{pH} = \text{pK}'a + \log \sqrt{2}$$

which is pH 6.3 for the carbonates and pH 7.0 for the phosphates.

The molal calcion buffer value at pH 7.35 is 0.111 or 28 per cent of the maximum for the carbonates and 0.265 or 45 per cent of the maximum for the phosphates.

The calcion buffer value of the carbonates of normal blood serum at pH 7.35 is  $3.5 \times 10^{-3}$  and that of the serum phosphate is  $0.5 \times 10^{-3}$ . The combined calcion buffer value of blood serum is  $4.0 \times 10^{-3}$ .

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### Preliminary report on human skin reactions to the "residue antigen" of the tubercle bacillus and to purified allied substances.

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Intracutaneous tests were made with solutions of the "residue antigen" prepared from dried tubercle bacilli, as described by Zinsser. This "antigen" shows none of the usual color reactions for the presence of protein. It has been shown by Zinsser and his co-workers to give specific precipitin tests with immune rabbit serum, and specific skin reactions of the tuberculin type in guinea pigs.<sup>1</sup>

Patients were tested with both O.T. and "residue antigen". We can conclude that the "residue antigen" from the tubercle bacillus is not injurious to the skin of the normal human being in amounts which cause injury to the skin of the individual allergic to the tubercle bacillus. This allergy of the infected individual is manifest as skin sensitiveness of varying degrees which parallels the sensitiveness to O.T.\* We have no evidence of any correlation of the degree of sensitivity with the extent or activity of the infection.

<sup>1</sup> Zinsser, H., and Barker, *J. Exp. Med.*, 1923, xxxvii, 275.

\* Roughly 0.0007 mg. of "residue antigen" is equivalent to 0.01 of O.T.